DEVELOPMENT OF POROUS HYDROXYAPATITE FOR ORTHOPEDIC APPLICATIONS

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BIJAY KUMAR DEBATA

(Roll No. 110BM0004)

Department of Biotechnology and Medical Engineering
National Institute of Technology Rourkela
Rourkela, Odisha -769008
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NATIONAL INSTITUTE OF TECHNOLOGY

CERTIFICATE

This is to certify that the thesis titled, **"Development of Porous Hydroxyapatite for Orthopedic Applications"** submitted by **Bijay Kumar Debata (Roll No. 110BM0004)** in partial fulfillment of the requirements for the award of **BACHELOR OF TECHNOLOGY** in **BIOMEDICAL ENGINEERING** at **National Institute of Technology, Rourkela** is an original work carried out by him under my supervision and guidance.

The matter embodied in the thesis has not been submitted to any University/ Institute for the award of any Degree.

Rourkela

12/05/2014

Dr. A. Thirugnanam

Assistant Professor

Department of Biotechnology and Medical Engineering

National Institute of Technology Rourkela

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110BM0004

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ABSTRACT

Hydroxyapatite (HA) is a calcium phosphate based ceramics which is known to be one of the most important implantable biomaterials due to its biocompatibility and osteoconductive properties. Porous HA implants have been used as substitutes for hard tissues in the clinical applications. The current work describes the synthesis of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] by the solid state reaction of β- tricalcium phosphate (β-TCP) and calcium hydroxide [$Ca(OH)_2$]. Both the chemicals were taken in the molar ratio of 3:3 and were sintered at two different temperatures for different time periods. For one sample, sintering was carried out at 1000^oC for 8hours and for the other one at 1150^oC for 2hours. The phase purity of both the samples was determined by XRD analysis and for 1000^0C higher phase purity was obtained. Then pellets of hydroxyapatite with 0% and 30% porosity were synthesized by implementing three different pore formers (starch, wheat flour and coffee) while sintering. The porous pellets were characterized in terms of mechanical and biological properties. Apparent porosity, bulk density, linear axial shrinkage, linear radial shrinkage and volumetric shrinkage were determined. Biodegradation study was carried out in Tris-HCl buffer solution at 37^0C having a pH of 7.4 by soaking the samples in the solution for 21 days. At the end of 21days, the final weights of the samples and change in pH of the solution was observed. From the observations the % weight loss was determined and release of calcium and phosphate was confirmed due to the increase in pH of the solution.

Keywords: hydroxyapatite, sintering, porous, shrinkage, biodegradation

CONTENTS

1. Introduction

1.1 Orthopedic Implants

In the last 50 years, a lot of advancement has been made in the field of biomaterials where materials required for biomedical applications have passed through three different generations, which are first generation, second generation and third generation biomaterials. The first generation biomaterials are bioinert materials, the second generation materials are bioactive and biodegradable materials and the third generation materials are the biomaterials which are designed in order to stimulate some specific responses at the molecular level.

Bone is at a steady state of rebuilding with osteoblasts transforming and mineralizing new bone network, osteocytes keeping up the framework and osteoclasts resorbing the grid. Bone coating cells are latent cells that are accepted to be antecedents for osteoblasts. Material properties depend basically on the way of the biomaterial and the manufacture process. Acknowledging all these components, the permeable hydroxyapatite is fit for emulating the characteristic hard tissue. Along these lines, it might be utilized as a suitable biomaterial for orthopedic requisition.

For the cells to develop into the pores and supplements of the cell to stream inside the pores-

- the width of the pores should not be under a specific quality which in ideal case is between 150 to 500µm.
- in request to go about as a resorbable material, the divider thickness ought not surpass a greatest quality for permitting the osteoblasts to resorb the material and to empower the osteoblast to supplant it by the own bone material of the body.

The bone recovery component includes the preceding steps:

- release of collagen atoms by the osteoblast cell to structure strands
- the osteoblasts combine calcium phosphate nanocrystals which are acknowledged as brushites and they are supplied from the framework vehicle
- from the network vesicles calcium phosphates are discharged and they help the epitaxial development of hydroxyapatite nanocrystals on collagen molecules.

1.2 Hydroxyapatite

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is a calcium phosphate based ceramics which is utilized as one of the most imperative implantable biomaterials because of its biocompatibility and osteoconductive properties. Hydroxyapatite (HA) has huge synthetic and physical likeness to the mineral constituents of human bones and teeth. It is a bioactive ceramics generally utilized as powders or within particulate structures in different bone repairs and as coatings for metallic prostheses to enhance their biotic properties. It has brilliant biocompatibility, bioactivity and osteoconduction properties. HA is thermodynamically the most steady calcium phosphate clay compound closest to the pH, temperature and organization of the physiological liquid. As of late, HA has been utilized for a mixture of biomedical provisions, including frameworks for pill discharge control. Because of the substance comparability between HA and mineralized bone of human tissue, manufactured HA shows solid proclivity to have hard tissues. Framing of synthetic bond with the host tissue offers HA a more excellent point of interest in clinical provisions over most other bone substitutes, for example, allograft or metallic inserts. HA has a hexagonal structure with a space bunch and cell measurements a=b=9.42 Å, and c=6.88 Å, where the space bunch alludes to a space bunch with a six-fold symmetry pivot with a three-fold helix and a micro plane. It has a careful stoichiometric Ca/P proportion of 1.67 furthermore is artificially very much alike to the mineralized human bone. Notwithstanding, in spite of compound similitudes, mechanical execution of manufactured HA is extremely poor contrasted with bone. Also, the bone mineral present a higher bioactivity contrasted with manufactured HA.

A standout amongst the most making a guarantee to methods to the issue of orthopedic implantation and harmed hard tissue repair is to have suitable biomaterial with upgraded mechanical and also natural properties. It is important to get fitting insert materials, with a specific end goal to encourage in-vitro and in-vivo tissue recovery and it ought to fulfill all the objectives which are needed for effective recovery, for example, osteoconductivity, and controlled debasement of the material, mechanical properties, formability, and so forth. Besides, one of the principle qualities that are needed in an orthopedic insert material is a high esteem of interconnected macroporosity of the material, so as to upgrade the cell colonization and stream transport of supplements and metabolic waste from the external environment to the embedded material. Around the conceivable materials utilized for implantation within orthopedic requisitions, calcium phosphates display a few points of interest, in view of their comparability and high similarity with characteristic bone.

Without further commotion, this field of biomedical building is an undeniably essential examination region whose objective is to surpass the restrictions of accepted medications focused around organ transplantation and other ordinary techniques for hard tissue repair. For getting the fruitful effect, the created material ought to have coveted mechanical and natural properties to have the capacity to copy the in-vitro conditions, the conveyance of tissue-inciting substances, for example, development and separation components to focus on areas, proper porosity keeping in mind the end goal to permit the developing cells in three-dimensional material.

This approach has seen huge examination advancements. Permeable grids, to which cells join and colonize, assume an indispensable part in incorporating bone–extracellular grid and cohorted organic particles to encourage the development of practical tissues/organs.

1.3 Why porous hydroxyapatite

Porous HA displays solid holding to the quick; the pores give a mechanical interlock prompting a firm obsession of the material. Bone tissue develops well into the pores, consequently expanding quality of the HA insert. The perfect bone substitute is a material that will structure a protected security with the tissues by permitting, and actually empowering, new cells to develop and enter. One approach to accomplish this is to utilize a material that is osteophilic and permeable, so new tissue, and eventually new bone, might be affected to develop into the pores and help to forestall extricating and development of the insert. A permeable hydroxyapatite covering encourages bone development through a very convoluted interface. At the point when pore sizes surpass 100 μm, bone develops through the channels of interconnected surface pores, along these lines keeping up the bone's vascularity and suitability. Since porous HA is more resorbable and more osteoconductive than thick HA, there is an expanding enthusiasm toward the advancement of manufactured permeable hydroxyapatite (HA) bone substitution materials for the filling of both burden bearing and non-load-bearing rigid deformities. Reenacting the human bone structure, porous HA platform has vast surface zone, which is advantageous for grip of organic tissue cell and development of new bone stage.

Porous HA inserts have served as bone substitute in the centers since long. HA with controlled porosity closely resembles the regular clay in the bone and is bioactive as in it is a non-harmful compound and interfacial bonds can create between HA and the living tissues prompting improved mechanical quality of the general structure. The porosity helps in tissue development and their coupling with the HA. On the

other hand, more level mechanical quality of immaculate HA has hampered its utilization as a bone insert material on account of clashing necessities of porosity and quality.

The need for porosity in bone recovery has been indicated by Kuboki et al. utilizing a rodent ectopic model and strong and permeable particles of hydroxyapatite for BMP-2(bone morphogenic proteins) conveyance; no new bone framed on the robust particles, while in the permeable platforms immediate osteogenesis happened. Further help hails from studies with metal permeable covered inserts contrasted with the non-covered material. Medicine of titanium compound insert surfaces with sintered titanium dabs made a permeable covering that improved cortical shear quality of the inserts recouped from sheep tibiae, while further covering with globules with hydroxyapatite did not bring about critical change. Titanium fiber-metal permeable coatings (45% porosity and 350μm normal pore size) amplified bone in development and expanded the potential for anxiety related bone resorption of femoral stems in a canine aggregate hip arthroplasty model.

1.4 Porous hydroxyapatite in orthopedic application

A key part in orthopedic provision for creation of insert materials is the decision of suitable biomaterial that serves as a format for cell associations and the establishment of bone-extracellular lattice to give structural backing to the recently framed tissue. Biomaterial for orthopedic implantation ought to meet certain criteria to serve this capacity, including mechanical properties like those of the bone repair site, biocompatibility and biodegradability at a rate equivalent with rebuilding. They serve basically as osteoconductive moieties, since new bone is stored by crawling substitution from nearby living bone. The material for osteogenesis ought to copy bone morphology, structure and capacity with a specific end goal to streamline mix into encompassing tissue. Bone is a structure made out of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) precious stones stored inside a natural network. The morphology is made out of trabecular bone which makes a permeable environment with 50–90% porosity. Four cell sorts are available in bone tissue: osteoblasts, osteoclasts, osteocytes and bone covering cells.

1.5 Objectives

The present study focuses on the following objectives-

- To synthesize hydroxyapatite by solid state reaction method
- To characterize synthesized hydroxyapatite powder by XRD analysis
- To fabricate porous hydroxyapatite pellets with different porosity using different pore formers
- To characterize the mechanical properties (linear and volume shrinkage, apparent porosity, bulk density) of porous HA pellets
- To characterize the biological properties (biodegradation) of porous HA pellets

2. Literature Review

2.1 Synthesis of hydroxyapatite

R. Ramachandra Rao *et al*. studied the strong state amalgamation and warm strength of HAP and HAP+β-TCP composite ceramic powders where biphasic composite mixture of HAP + β-TCP were ready with monetarily accessible tricalcium phosphate (TCP) and calcium hydroxide [$Ca(OH)_2$] [1]. These reactants were blended in the molar proportions running from 3:0 to 3:4 in deionized water, processed and slip-cast into plates which were then hotness treated in the temperature reach of 600°C to 1250°C. The items shaped were described by X-Ray beam diffraction (XRD) and I-R spectroscopic methods for identification of stages framed and useful gatherings display in them. While tricalcium phosphate and calcium hydroxide taken in the molar proportion of 3:2 and 3:3 brought about immaculate HAP when high temperature treated at 1000°C for 8h, the 3:1 and 3:1.5 molar degree structures brought about a biphasic mixture of HAP + β-TCP for comparable hotness medications. Heat medicine of 3: 4 molar proportion organizations of tricalcium phosphate and calcium hydroxide at 1000°c yielded HAP with free CaO as the optional stage. This system is suitable for the arrangement of immaculate stoichiometric β-TCP, or HAP and also their biphasic (HAP + β-TCP) composite powder mixtures.

S.-H. Rhee considered the blend of hydroxyapatite through mechanochemical medicine where Hydroxyapatite powder was integrated with calcium pyrophosphate ($Ca_2P_2O_7$) and calcium carbonate ($CaCO_3$) through robust state response [2]. The two powders were blended in $(CH_3)_2CO$ and water, individually, and the single period of hydroxyapatite was seen to happen just in the powder processed in water, without the extra supply of water vapor throughout high temperature medication at 11000c for 1 h. The outcomes were demonstrated as far as the mechanochemical response that could supply enough measure of hydroxyl gathering to the beginning powders to structure a solitary period of hydroxyapatite. Useful significance of the effects is that the powder of high crystalline hydroxyapatite might be acquired by the straightforward processing in water and consequent heat treatment.

K.C.B. Yeong *et al.* examined the mechanochemical combination of hydroxyapatite in which a solitary stage hydroxyapatite of high crystallinity was discharged by more than 20h of mechanical actuation of a dry powder mixture of calcium oxide (CaO) and anhydrous calcium hydrogen phosphate ($CaHPO_4$) without further warm medication at high temperatures [3]. The ensuing hydroxyapatite powder showed a normal molecule size of and 25nm and a particular surface territory of 76.06 m^2/g, as measured by multi-point BET method. It was sintered to a thickness of 98.20% hypothetical thickness at 1200^0C for 2 h.

R. Ramachandra Rao considered the amalgamation and sintering of hydroxyapatite–zirconia composites in which hydroxyapatite (HA) has been blended in vicinity of 10–30 wt.% of m-ZrO_2 by strong state response between tricalcium phosphate (TCP) and $Ca(OH)_2$ at 1000^0C for 8h [4]. The m-ZrO_2 was halfway changed over into t-ZrO_2 by incomplete utilization of Cao which thusly brought about a mixture of h-TCP and HA. On sintering these HA–h-TCP–ZrO_2 composite powders at 1100–1400^0C for 2h, the HA is further decayed into h-TCP and CaO. The CaO so generated responds further with m-ZrO_2/t-ZrO_2 producing a mixture of t-Zro2 and $CaZrO_3$ in diverse extents. These different stages framed meddle with the sinterability of the composites because of their differential shrinkages prompting a general decreased thickness as contrasted with that of immaculate HA. The composites demonstrate a T-onset of deterioration at around 1150^0C and a 40% HA yield was acquired at the most noteworthy sintering temperature of 1400^0C. The items were subjected to XRD for stage dissection and the microstructural characteristics were examined by SEM.

Layrolle *et al.* studied the sol–gel combination of formless calcium phosphate and sintering into microporous hydroxyapatite bioceramics where another course for get ready hydroxyapatite bioceramic has been depicted [5]. A formless, nanosized, and carbonate-holding calcium phosphate powder that had a Ca/P ratio of 1.67 was combined from calcium diethoxide and phosphoric corrosive in ethanol by means of a sol–gel system. The powder was pressed at 98 MPa into green examples and after that warmed to a temperature extent of 500°–1300°C. At 600°C, the powder solidified to a carbonated hydroxyapatite and a hint of b-tricalcium phosphate before changing over to hydroxyapatite at 900°C. The warm crystallization was connected with grain development, shrinkage, and dynamic surface dispersion. The initiation vitality of grain development was 37±2 kJ/mol. In the wake of sintering at 1100°C, the deterioration of carbonated hydroxyapatite produced a microporous ceramic with a normal

pore size of 0.2 μm and an open porosity of 15.5%. This microporous bioceramic might be used as bone filler.

2.2 Fabrication of Porous hydroxyapatite

K. Lim *et al.* examined on the arrangement of macroporous calcium silicate earthenware production by utilizing poly ethylene glycol (PEG) as pore previous [6]. Sintered minimal with porosity in the range of 40-75% have been acquired by changing the sum and size of fired and PEG particles and the sintering temperature. Sub-atomic weight of PEG plays an essential part in the morphology, structure and the pore size of the microporous calcium silicate. The PEG plays a primary part in bigger pore framing, when enough mass of PEG with easier sub-atomic weight were included. The accessible substance of PEG added substance in the mixture result need to lessening on account of bigger atomic weight of PEG than that of more diminutive sub-atomic weight of PEG as indicated by the level of miscibility.

Xiaohong Yang and Zhihong Wang studied the synthesis of biphasic ceramics of hydroxyapatite and b-tricalcium phosphate with controlled stage substance and porosity where biphasic permeable pottery of hydroxyapatite and b-tricalcium phosphate [7]. The procedure was focused around strong state responses of brushite with calcium carbonate. The ideal high temperature medicine conditions for generating unadulterated stoichiometric HA, b-TCP and a biphasic composite of HA and b-TCP with unmistakable stage synthesis were recognized by the strategies for warm dissection, X-beam diffraction and IR retention spectroscopy. The ideal high temperature medication conditions are sintering for 2 h at 1200 °c for HA and sintering for 2h at 930°C for b-TCP. Biphasic composites of HA and HA stage b-TCP with controlled stage organizations might be gotten by modifying the sintering temperature from 1000–1100°C. As a gas framing executor, a certain measure of uncalcined powder was blended with the comparing precalcined powder to handle permeable earthenware production with diverse microporosities. Froth ceramics with macropores have been ready by a dipping procedure. The pore structure was analyzed utilizing examining electron microscopy.

2.3 Pore formers

Z. Zivcova *et al*. concentrated on the alumina earthenware production ready with new pore-structuring executors where the utilized natural pore framing operators (PFAs) for creation of permeable frameworks

of alumina ceramics [8]. They have taken natural pore shaping operators on the grounds that they are non-poisonous to biotic environment and these might be vanished at a more level temperature throughout sintering which will prompt the framing of compelling pore structure. Likewise, natural pore formers are of living inception and could be utilized to control porosity, pore size and pore shape. Their work concerned the characterization and testing of a few less basic pore-framing operators like lycopodium, espresso, flour and semolina, poppy seed which are of potential enthusiasm from the perspective of size, shape or accessibility. The PFAs researched in this work are in the size extent from 5µm (rice starch) to give or take 1mm (poppy seed), all with pretty much isometric shape. The burnout conduct of PFAs is considered by warm dissection, i.e. thermogravimetry and differential thermal analysis.

Almirall *et al.* examined the advancement of the new advances of bone tissue building requires the creation of bioresorbable macroporous platforms [9]. Calcium phosphate bonds are great applicant materials for the advancement of these frameworks, as an elective to the conventional permeable sintered pottery. In this work a novel two-stage system, situated in the frothing of an a-tricalcium phosphate (a-TCP) concrete glue and its consequent hydrolysis to a calcium lacking hydroxyapatite (CDHA) are introduced. The frothing executor was a hydrogen peroxide (H_2O_2) result, which disintegrates in water and oxygen gas. CDHA froths, which joined an interconnected macroporosity with a high microporosity were gotten. The apatitic stage got by the hydrolysis response was more like the biologic one, regarding substance synthesis, crystallinity and particular surface than the hydroxyapatites acquired by sintering. The rate of porosity in the froths arrived at a 66%. It was indicated that it was conceivable to control the porosity, pore size and shape by distinctive preparing parameters, for example, the fluid to-powder degree, the centralization of the H_2O_2 result and the molecule size of the powder.

Yang Lie *et al*. considered the permeable pottery ready from nano estimated hydroxyapatite powders by including water solvent and water insoluble starch [10]. The outcomes demonstrated that little pores of a few micrometers or less could be generated by including water dissolvable starch as a pore previous. Two sorts of starch have diverse pore structuring systems. The porousness of permeable pottery could be significantly enhanced by adding the insoluble starch to channel the little pores instead of singularly utilizing water dissolvable starch. The control of porousness could be accomplished by altering the substance degree of the water solvent starch to water insoluble starch. Quality tests demonstrated that the

pottery have rather higher quality. Thus a sort of permeable separating materials with little pores, controllable penetrability and great quality could be ready by utilizing starch added substances.

2.4 Biodegradation of porous hydroxyapatite

Ashok Priya *et al.* contemplated the in vitro disintegration of calcium phosphate-mullite composite in mimicked body liquid where they have created novel cap-mullite composites for bone insert provisions [11]. Keeping in mind the end goal to acknowledge such requisitions, the *in vitro* disintegration conduct of these materials was required to be assessed. In this viewpoint, their examination work reported the disintegration conduct of immaculate hydroxyapatite (Hap) and hydroxyapatite composites with 20–30 wt% mullite in mimicked body liquid (SBF). The in vitro disintegration, tests were done for diverse time terms beginning from 7 days up to 28 days. XRD and SEM effects indicated just about no disintegration for immaculate Hap and Hap composite with 30wt% mullite. Then again, Hap-20 wt% mullite composite showed extensive disintegration following 7 days. The a-TCP stage basically helped the disintegration process. In light of the element changes in pH, ionic conductivity, Ca and P particle focus in SBF and microstructural perceptions of the bioceramic surfaces after different time periods of submersion in SBF, the contrasts in disintegration conduct were examined.

Hyun-Min Kim *et al*. contemplated the process and energy of bone like apatite framing on sintered hydroxyapatite in a reenacted body liquid [12]. The surfaces of two hydroxyapatites (HA), which have been sintered at distinctive temperatures of 800 and 1200^0C, was examined as a capacity of absorbing time reenacted body liquid (SBF) utilizing transmission electron microscopy (TEM) appended with vitality dispersive spectrometry (EDX) and laser electrophoresis spectroscopy. The HA uncover negative surface accuse and consequently communicate of the positive calcium particles in the liquid to structure the Ca-rich undefined calcium phosphate (ACP), which picks up positive surface charge. The Ca-rich undefined calcium phosphate (ACP) on the HA then collaborates with the negative phosphate particles in the liquid to structure the Ca-poor ACP, which balances out by being solidified into bonelike apatite. This procedure of apatite development was indicated to be languid on the HA sintered at higher temperature. This wonder is ascribed to introductory more level negative surface charge of the HA sintered at higher temperature owing to a destitution in surface hydroxyl and phosphate bunches, which are answerable for the surface cynicism. The procedure and energy of apatite creation on HA could be

influenced by mass elements, for example, thickness and surface zone and in addition by surface components, for example, arrangement and structure. The iso-electric purpose of HA in water is at ph going between 5 and 7, and is easier than the ph of the SBF (7.4).

Hence on inundation in SBF, the HA could uncover negative surface charge by uncovering hydroxyl and phosphate units in its gem structure. The HA surface uses this negative accuse to interface particularly of the positive calcium particles in the liquid, thus framing a Ca-rich ACP. The development of the Ca-rich ACP is accepted to happen in successive gathering of the calcium particles, which makes the Ca-rich ACP procure and increment positive charge. The Ca-rich ACP on the HA in this way connects particularly with the negative phosphate particles in the liquid to structure a Ca-poor ACP. This sort of Ca-poor ACP has been seen as a forerunner, which in the long run takes shape into bonelike apatite on different bioactive earthenware production.

Han Guo *et al.* considered biocompatibility and osteogenicity of degradable Ca-insufficient hydroxyapatite frameworks from calcium phosphate bond for bone tissue building by a molecule filtering strategy [13]. The morphology, porosity and mechanical quality and in addition debasement of the frameworks were portrayed. The outcomes demonstrated that the CDHA frameworks with a porosity of 81% indicated open macropores with pore sizes of 400–500μm. Thirty-six for every penny of these CDHA platforms were debased following 12 weeks in Tris–HCl result. The effects uncovered that the CDHA frameworks were biocompatible and had no negative consequences for the mesenchymal foundational microorganisms (MSCs) *in-vitro*. The *in-vivo* biocompatibility and osteogenicity of the platforms were researched. The CDHA framework following 8 week implantation demonstrates great biocompatibility and broad osteoconductivity.

3. Materials and Methods

3.1 Solid state synthesis of hydroxyapatite

Hydroxyapatite was synthesized by the solid state reaction of β-tricalcium phosphate (β-TCP) and calcium hydroxide [$Ca(OH)_2$]. The first step in the synthesis was to take the two starting materials in 3:3 molar ratio, then mix and grind them well by the help of a mortar and pestle. The grinding was done in order to get a homogeneous distribution of both the reactants. Two such samples were prepared grinding them till 1h. The stoichiometric calculations involved,

Molecular weight of $Ca(OH)_2$ = 74.093 g/mol

Molecular weight of $Ca_3(PO_4)_2$ = 310.18 g/mol

So, 3M of TCP = 310.18g x 3 = 930.54g

3M of $Ca(OH)_2$ = 74.093g x 3 = 148.186g

Dividing both the values by 1000, it was obtained that

TCP = 0.93g x 2 =1.86g

$Ca(OH)_2$ = 0.22g x 2 = 0.44g

Fig 3.1 Grinding of the β-TCP and $Ca(OH)_2$ in mortar and pestle

Hence, two samples of the homogeneous mixture of TCP and $Ca(OH)_2$ were prepared taking 1.86g of TCP and 0.44g of $Ca(OH)_2$ for each sample. Thus we obtained two samples in the molar ratio of 3:3. The next step of the synthesis involves sintering both the samples separately at two different temperatures for two different time periods. One of the samples was sintered at 1000^0C for 8h whereas the other one was sintered at 1150^0C for 2h. The powders were held in molds and then heated to the above mentioned temperatures which are below their melting points. The atoms in the powder particles diffused across the boundaries of the particles, fusing the particles together leading to the formation of hydroxyapatite.

3.2 Characterization of hydroxyapatite

The hydroxyapatite powder synthesized by the above mentioned method was characterized by phase analysis in XRD analysis in order to observe the composition of the powder and verify the phase purity of hydroxyapatite. XRD analysis was carried out within the scanning range of 20-80˚ with scanning rate 200/min and step size 0.05 using the room temperature powder X-ray diffraction with Cu Kα radiation. The HA peaks were identified by referring JCPDS file (reference 74-0565).

3.3 Fabrication of porous hydroxyapatite pellets:

In order to make porous HA pellets, three different pore formers were identified which were easily available. These pore formers are coffee, wheat flour and starch. Samples of 0% and 30% porosity were prepared by weighing required amount of hydroxyapatite and pore former and mixing them well by grinding in mortar and pestle. For 0% porosity amount of HA taken was 0.6g for each sample without any pore former adding in it. Similarly for 30% porosity 0.42g of HA and 0.18g of pore former were taken. Four sets of samples for each porosity were prepared and a total 16 samples were obtained. After weighing all the samples, 5% PVA binder was prepared.

Table 3.1 Three different pore formers, their boiling points and resulting pore sizes

Pore former	Boiling point(^{0}C)	Resulting pore size(µm)
Starch	250	5
Wheat flour	290-350	<100
Coffee	250-550	<100

Table 3.2 Amount of HA and pore former for different porosity

Porosity (%)	HA (g)	Pore former(g)
0	`0.6	0
30	0.42	0.18

Preparation of 5% PVA binder:

In order to enhance the binding ability of the hydroxyapatite powders with the pore former a binder having boiling point below the melting point of the hydroxyapatite powder is chosen. PVA 5% serves as an effective binder in this case. For preparing 5% PVA binder, 50ml of water was taken in a 100ml glass beaker and was stirred using a magnetic stirrer at 450rpm maintained at 70^{0}C. When the water became warm 2.5g of PVA was added little by little into the water. The stirring was carried out till 2h. After that, the solution was placed inside a 50ml centrifuge tube and it was covered tightly in order to avoid its contact with air.

Fig 3.2 Preparation of PVA Binder

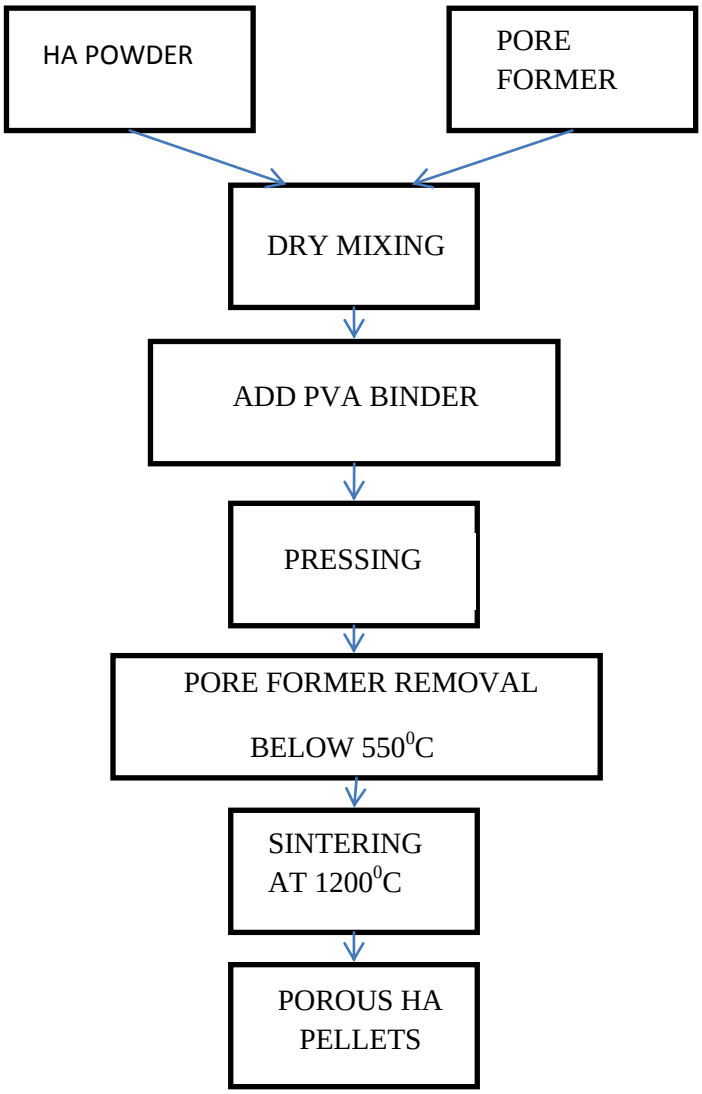

Fig 3.3 Flow chart for fabrication of porous HA pellets

3.4 Characterization of porous hydroxyapatite pellets

The sintered hydroxyapatite pellets using different pore formers in the form of pellets were characterized in terms of mechanical and biological properties. The characterization was done as follows:

3.4.1 Mechanical properties

The mechanical properties of the porous pellets of hydroxyapatite were investigated. These mechanical properties involved linear shrinkage, volumetric shrinkage, apparent porosity measurement, bulk density and relative porosity measurement.

3.4.1.1 Linear and volume shrinkage measurement

During the sintering of the compact samples made from the hydroxyapatite powder and pore former, the reduction of pore volume results in shrinkage. In general the shrinkage is distinguished in three types.

(a)Volume shrinkage- This occurs due to the densification during sintering

(b)Axial shrinkage- This is linear shrinkage in the direction of pressing or in the axial direction

(c)Radial shrinkage- This is linear shrinkage at right angles to the direction of pressing or in the radial direction

In order to determine both linear and volume shrinkage, the diameter, thickness and volume of each sample were measured before sintering and after sintering by the help of a digital vernier caliper. While taking the measurements of the samples before sintering, care must be taken during the contact of the vernier caliper with the sample as the sample had poor strength and might result in breakage of its edges.

From the obtained data we calculated the linear and volume shrinkage as,

$$\textbf{Axial Linear shrinkage} = \mathbf{h_1 - h_2}$$

$$\textbf{Radial linear shrinkage} = \mathbf{D_1 - D_2}$$

$$\textbf{Volume shrinkage} = \mathbf{V_1 - V_2}$$

where,

h_1=thickness of the sample before sintering

h_2=thickness of the sample after sintering

D_1=diameter of the sample before sintering

D_2=diameter of the sample after sintering

V_1=volume of the sample before sintering

V_2=volume of the sample after sintering

3.4.1.2 Porosity measurement

Porosity of the sintered HA pellets was measured by applying Archimedes' principle. At first the dry weights of the samples were taken. Then the samples were kept inside a beaker filled with kerosene and it was kept inside a desiccator for half an hour. This was done to make the kerosene enter inside the pores of the scaffold. When bubbles start coming from the sample surface, it was taken out. The suspended as well as soaked weight of samples was taken. Before taking the soaked weight, the surfaces of the sample were wiped gently by a tissue paper soaked in kerosene.

Apparent porosity and bulk density of the samples were represented as:

$$\textbf{Aparent porosity} = \frac{\mathbf{S - D}}{\mathbf{S - H}}$$

$$\textbf{Bulk density} = \frac{\mathbf{D \times Density\ of\ kerosene}}{\mathbf{S - H}}$$

$$\textbf{Relative density} = \frac{\textbf{Bulk density}}{\textbf{Theoretical density}} \times \mathbf{100}$$

$$\textbf{Porosity} = \mathbf{1 - Relative\ density}$$

where,

D = dry weight

Density of kerosene = 0.84 g/cc

Theoretical density of HA = 3.16 g/cc

S = suspended weight

H = soaked weight

Density of the available kerosene was determined by the following method:

In a small beaker, 20ml of kerosene was taken and then the weight of the kerosene was determined by help of the weighing balance which was 16.8g.

$$\text{density} = \frac{\text{mass}}{\text{volume}} = \frac{16.8}{20} = 0.84\text{g/ml}$$

3.4.2 Biological characterization

3.4.2.1 Biodegradation of porous HA pellets

Biodegradation test of porous HA pellets were carried out by taking Tris-HCl buffer solution. 0.1M Tris-HCl solution was prepared by using distilled water. At first, 2.42g of Tris was added to 150ml of distilled water. Then the pH of the solution was maintained 7.4 at 37^0C by adding 1M HCl. After that the solution volume was made 200ml by adding the rest amount of water. Initial weights of the samples were taken. Then each sample was soaked in 5ml of Tris-HCl buffer solution in incubator at 37^0C for 21days. After 21 days they were removed carefully and dried. Then the final weights of samples were taken.

The Tris-HCl buffer solutions were taken out and pH of each sample solutions were determined by the help of digital pH meter. Then the Tris-HCl buffer solutions were observed separately under the UV-spectrometer and the peak wave lengths and absorbance values were noted down for each sample. From these values the released components and their amount of release were investigated.

$$\textbf{\% weight loss} = \frac{w_0 - w_t}{w_0} \times 100$$

where,

w_0 = initial weight of sample

w_t = final weight of sample after soaking in Tris-HCl solution

4. Results and Discussion

4.1 Characterization of hydroxyapatite

The prepared HA powder samples were identified by performing XRD analysis. Fig-4.1 shows the XRD pattern of the sample sintered at 1000^0C and Fig-4.2 shows the XRD pattern of the sample sintered at 1150^0C. The peaks of hydroxyapatite were identified referring to the JCPDS file (reference 74-0565) and the purity of both the samples was compared.

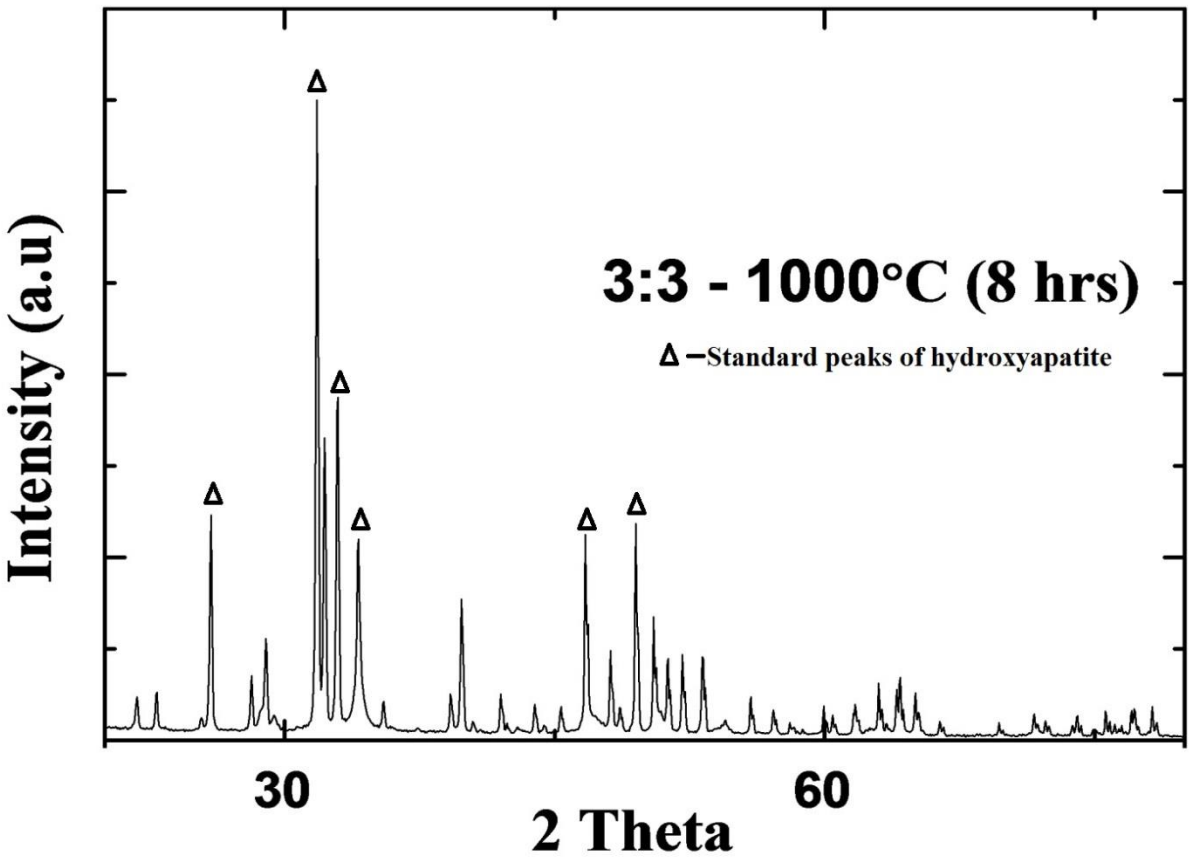

Fig-4.1 XRD pattern of the powdered mixture consisting of β-TCP and $Ca(OH)_2$ in the ratio 3:3 sintered at 1000^0C for 8h

The peaks were identified at 31.77^0, 32.19^0, 32.9^0, 33.8^0, 42.6^0 and 48.5^0 which corresponded to the standard peaks of hydroxyapatite. Along with these peaks some other peaks were also observed which were because of the presence of impurities like CaO and TCP.

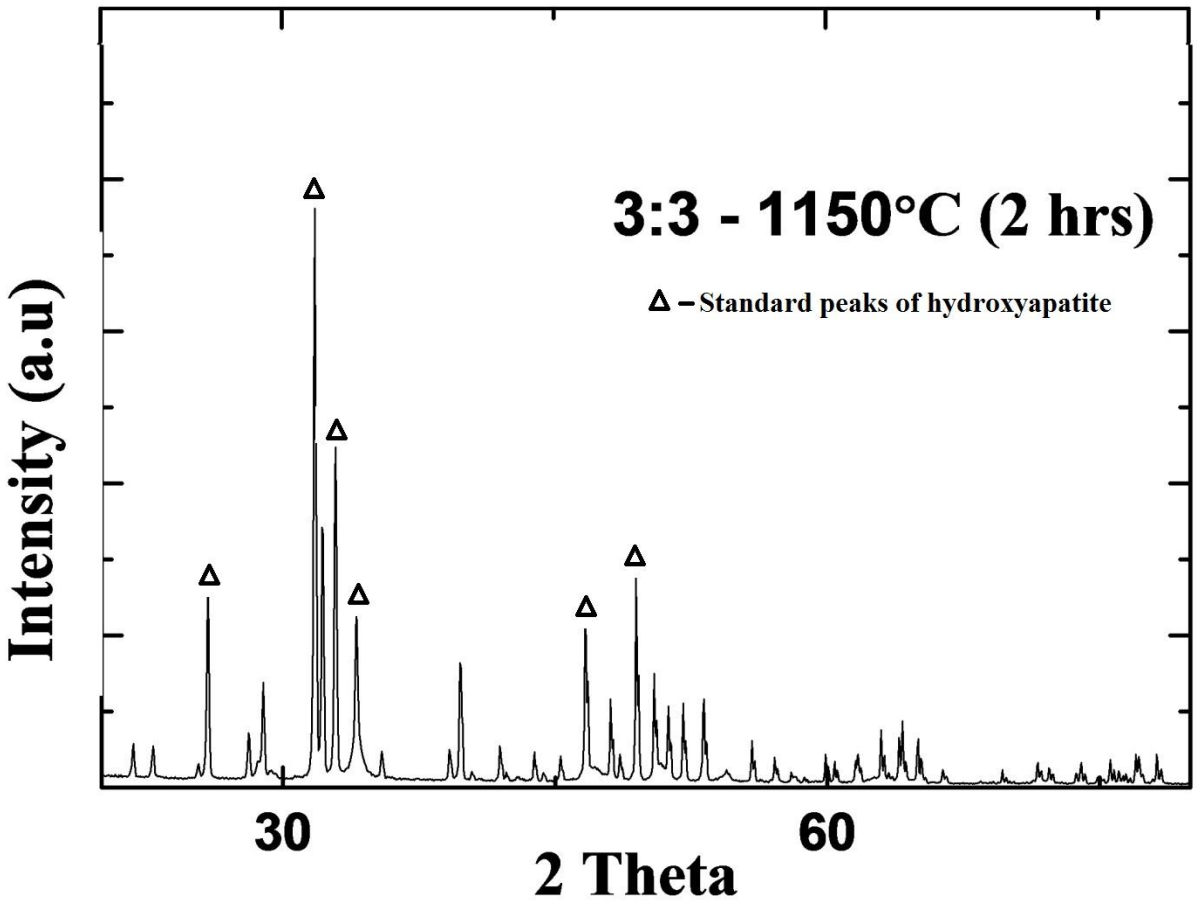

Fig-4.2 XRD pattern of the powdered mixture consisting of β-TCP and $Ca(OH)_2$ in the ratio 3:3 sintered at 1150^0C for 2h

After comparing the phase analysis of both the samples, the sample with 3:3 ratio of β-TCP and $Ca(OH)_2$ sintered at 1000^0C was found to have a phase purity of 88% whereas the other one was found to have phase purity of 86%. So the first sample was taken for bulk production as the phase purity in this case was higher compared to the other sample. Keeping the same molar ratio of 3:3, 51.5g of β-TCP and 12.21g of $Ca(OH)_2$ were taken for bulk production and the same steps were repeated again resulting in 60g of HA.

4.2 Characterization of porous hydroxyapatite pellets:

Four sets of HA pellets were obtained after compacting and sintering of the mixture of HA and pore former which are shown in the figures below. The sample without any pore former was dense and the other samples which were fabricated using pore former were porous in nature.

Fig 4.3 HA pellets fabricated without any pore former (0% porosity)

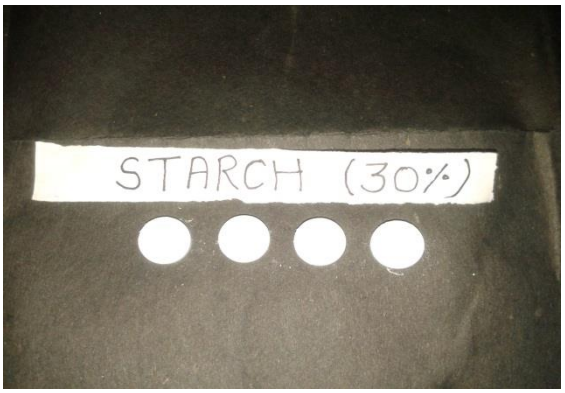

Fig 4.4 HA pellets fabricated using starch as pore former (30% porosity)

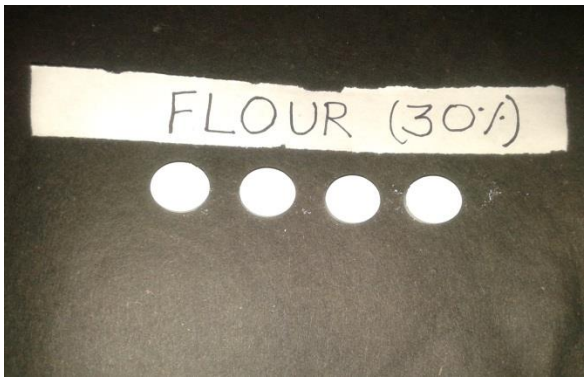

Fig 4.5 HA pellets fabricated using flour as pore former (30% porosity)

Fig 4.6 HA pellets fabricated using coffee as pore former (30% porosity)

The characterization of the dense as well as porous HA pellets was performed by means of mechanical and biological properties as follows:

4.2.1 Mechanical properties

4.2.1.1 Linear and Volume shrinkage

The linear and volume shrinkage of all the samples were obtained by the help of digital vernier caliper. Linear shrinkage included both axial and radial shrinkage of the scaffolds. Table-4.1 shows the values of linear and volume of shrinkage of the pellets which were determined by taking the average over the four samples for different pore former. In case of volume shrinkage the dimension was changed from mm^3 to cubic centimeter to obtain the values as given below.

Also the sintered density was determined from the values of weight of the samples after sintering and the new volume of the pellets. The density before sintering is known as green density of the samples and it was observed that the density after sintering was always more than the density before.

Table-4.1 Linear and volume shrinkage of the pellets

Sample (porosity)	Pore former used	Linear shrinkage (mm)		Volume shrinkage (cc)
		Axial shrinkage	Radial shrinkage	
HA (0%)	No pore former	0.09	0.65	0.042
HA (30%)	Wheat flour	0.16	0.56	0.050
HA (30%)	Starch	0.24	0.65	0.066
HA (30%)	Coffee	0.18	0.77	0.060

It is evident from the above result that the final thickness, diameter and volume of the samples after sintering is less than that before sintering which indicates shrinkage in the samples. The axial shrinkage as well as volume shrinkage of the pellets fabricated using starch as pore former is more compared to the other pellets. Similarly, radial shrinkage was found to be more when coffee was used as pore former.

4.2.1.2 Apparent porosity and bulk density

The apparent porosity and bulk density values determined by the Archimedes' principle are shown in the Table-4.2 below. These results were obtained by taking the average over 3 sets of samples for each pore former.

Table-4.2 Apparent porosity and bulk density of the pellets

Sample (porosity)	Pore former used	Apparent porosity (%)	Bulk density
HA (0%)	No pore former	4.88	2.38
HA (30%)	Wheat flour	32.34	1.45
HA (30%)	Starch	34.0	1.41
HA (30%)	Coffee	33.58	1.44

As it is clear from the above table, in all the cases, the apparent porosity was found to be more than the desired porosity. In case of 0% porosity, it is 4.88% and in case of 30% porosity, it was found to be 32-34%. The change in the desired porosity value can be explained considering the fate of the PVA binder. While sintering it also evaporates from the sample along with the pore former. In addition to this, the effect of high temperature also plays a significant role for porosity of the samples.

4.2.2 Biological properties

4.2.2.1 Biodegradation of HA pellets

The characterization of the fabricated porous pellets of HA was carried out in terms of biodegradation and the % weight losses as well as change in pH of the Tris-HCl buffer solution were observed. Table-4.3 shows these results which are taken for four different samples.

Table-4.3 Weight loss of the samples and pH of the final Tris-HCl solution

Sample (porosity)	Pore former used	% weight loss	pH of the final Tris-HCl solution
HA (0%)	No pore former	3.2	8.2
HA (30%)	Wheat flour	5	9.1
HA (30%)	Starch	3.1	8.5
HA (30%)	Coffee	4.8	8.9

As it is evident from the above table, pH of all the solutions is different. In the beginning, while putting the samples into the Tris-HCl buffer solution the pH was adjusted to 7.4. From the current observation, it was found that there is an increment in the pH of the solutions in each case which indicate that the solution has become more basic in nature. This is because of the release of calcium and phosphate ions from the pellets into the solution. As calcium is basic in nature, it forms $Ca(OH)_2$ when released from the pellets and thus gives rise to a more basic solution. The calcium phosphate release was found to be more in case of HA pellets fabricated using wheat flour as pore former. At the same time, the weight loss of HA pellets fabricated using wheat flour as pore former was found to be more when compared to the other pore former samples which conforms the highest degradation of those samples.

5. Summary and Conclusions

Conclusions:

- Two samples of hydroxyapatite (HA) were prepared successfully from β-tricalcuim phosphate (β-TCP) and calcium hydroxide ($Ca(OH)_2$) in the ratio of 3:3 by solid state reaction method, one sintered at 1000^0C for 2h and 1150^oC for 8h.
- The phase analysis of both the samples was performed in XRD analysis and it was found that the sample sintered at 1000^oC had higher purity (88%) compared to the one sintered at 1150^oC. So it was taken for bulk production.
- Three pore formers wheat flour, starch and coffee were identified having boiling point lower than the melting point of HA. The forming pore size obtained was found to be less than 100μm
- Porous pellets of hydroxyapatite were prepared by sintering the compacted pellets of HA and pore former at 1200^0C.
- The linear axial shrinkage, radial shrinkage and volume shrinkage of the porous pellets were determined. It was found that in case of starch the axial shrinkage was 0.242mm which is more compared to other samples and in case of coffee the radial shrinkage was 0.77mm which is more compared to the other sample. Volume shrinkage of all the samples was more or less same.
- The apparent porosity and bulk density of all the samples were determined by the Archimedes' principle and it was observed that the apparent porosity value was more than the desired porosity i.e. it was found 4.88% for 0% porosity and 32-34% for 30% porosity. Bulk density of dense HA pellets was found to be 2.38g/cc which was more compared to the porous pellets (bulk density ranging from 1.41-1.45g/cc)
- Biodegradation of all the samples were carried out in Tris-HCl solution having pH 7.4 at 37^oC for 21 days and an increment in the pH of the solutions was observed which was due to the release of calcium and phosphate ions from the pellets into the solution. The calcium phosphate release was found to be more in case of HA pellets fabricated using wheat flour as pore former.
- The weight loss of HA pellets fabricated using wheat flour as pore former was found to be more when compared to the other pore former samples.

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